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Summary

Asymptomatic excretion of group B streptococcus (GBS) in breast milk may be an under-recognised cause of neonatal and recurrent infection. We report the case of late-onset and recurrent infection in newborn twins resulting from ingestion of maternal breast milk infected with GBS. Genetic analysis of isolates is equally presented.

Key-words: group B streptococcus; breast milk; recurrent infection; genetic analysis; late-onset disease

Introduction

Group B streptococcus (GBS) is the most frequent cause of neonatal sepsis and meningitis. Most cases occur in the first week of life and are related to vaginal carriage in the mother (early-onset disease). Conversely, late-onset disease (between 1 week and 3 months of age) is less common and is hand-transmitted by nursery personnel or via other nosocomial or community pathways [6]. Late- onset and recurrent disease have also been reported with the ingestion of infected mother's milk, with some cases confirmed using molecular techniques [2-4, 7, 9, 13]. We report in this article the case of late-onset and recurrent infection in newborn twins resulting from ingestion of maternal breast milk infected with GBS. In this case genetic analysis demonstrated that all GBS isolates (maternal breast milk and vaginal isolates; twin CSF and blood isolates) were identical, but additional genetic analysis also revealed that the GBS isolates were of a particularly virulent clone belonging to serotype III, as are 90% of the strains responsible for late- onset disease [3].

Case report

Premature twins were delivered by spontaneous vaginal delivery at 31 weeks gestation, 48 hours following membrane rupture. The 26 year- old mother, gravida 1, para 2, received intrapartum antibiotic prophylaxis (single dose of penicillin G) due to positive vaginal GBS culture at 30 weeks gestation. Twin 1, female, weighed 1600g with an Apgar score of 10/10 at 1 and 5 minutes. No respiratory failure was noted and early enteral feeding was started with raw breast milk at day 1. Total enteral alimentation with breast milk was obtained at day 6. Twin 2, male, weighed 1720g with an Apgar score of 4/9 at 1 and 5

minutes. He was intubated shortly after birth due to respiratory failure and received one dose of surfactant. By the 3rd hour of life, he was extubated and nasal CPAP was initiated. Enteral alimentation with raw breast milk was introduced at day 4. Antibiotic therapy with cefotaxime and amoxicillin was prescribed for both infants due to incomplete prepartum antibiotic prophylaxis and stopped at day 2 due to negative C- reactive protein as well as negative gastric and blood cultures. Cerebral ultrasound examination was normal for both infants at day 4.

On day 13, Twin 2 developed cardio-respiratory instability and blood culture tested positive for group B streptococcus. Meningitis was suspected due to elevated CSF protein concentration. Until day 16 Twin 1 was asymptomatic with negative C- reactive protein control. On day 16, she developed respiratory distress and subsequent blood and cerebrospinal fluid cultures tested positive for GBS. Antibiotic treatment with amoxicillin at 200mg/day was prescribed for 14 days, in association with an aminoglycoside during the first 48 hours of treatment. Control blood cultures were negative after day 1, 3 and 5 of treatment. Cerebral ultrasound examination controls were normal for both infants. At day 41 of life Twin 1 developed septic syndrome with parotitis and was transferred to the NICU. Blood culture was positive for GBS. Cardiac ultrasound examination was normal. Antibiotic therapy with cefotaxime (200mg/kg/day) and tobramycin (5mg/kg/day) was initiated. At day 7 of infection tomodensitometry examination identified cerebral microabcess and modification of the antibiotherapy ensued, with the administration of ciprofloxacin (30 mg/kg/day) associated with cefotaxime (250 mg/kg/day) for 3 weeks. Then oral amoxicillin was initiated for 3 additional weeks. Mastitis was diagnosed in the infants' mother 24 hours following discovery of GBS infection in Twin 1 (day 42). Milk culture tested positive for GBS and the maternal infection was treated with amoxicillin for 10 days. Breastfeeding was

suspended and a 10- day preventive oral amoxicillin treatment given to the non- infected twin (confirmed via negative blood and CSF cultures as well as negative CRP controls). Following this infection, the infants remained on pasteurized breast milk. Follow- up at one year showed no cerebral anomalies upon ultrasound examination in association with normal neurological examinations at 1 year of life.

Analysis revealed all strains as belonging to serotype III. Epidemiological relationships between maternal and neonatal GBS isolates were investigated by pulsed-field gel electrophoresis (PFGE) of DNA restricted with *Sma*I [11]. Analysis was conducted on maternal vaginal and raw breast milk isolates (2 isolates), a single Twin 2 blood culture isolate (1 isolate), and on Twin 1 CSF and first- and -second blood culture isolates (3 isolates). All six isolates displayed identical PFGE patterns, revealing their genetic relationship (figure 1A).

Characterization of isolate virulence was conducted by multiplex PCR according to primers and method previously described [11, 12]. First, amplification of the tRNA gene clusters at the 3' end of rRNA operons produced a unique fragment of 1.2 Kb (figure 1B); second, *hyl*B amplification produced a 0.3 Kb fragment, showing no IS*1548* insertion within the gene (figure 1B). This pattern was correlated to the invasive phylogenetic division I defined by Musser *et al*. in multilocus enzyme electrophoresis (MLEE) analysis [8, 10].

Discussion

Cases reporting neonatal late and recurrent group B streptococcal disease associated with raw maternal milk are rare, and few are the studies in which

genetic evidence is proposed for this scenario [2, 7, 13]. In our case, not only was total DNA macrorestriction analysis conducted, showing indistinguishable patterns for the six isolates, but additional genetic analysis also revealed that the GBS isolates belonged to a particularly virulent clone shown to produce more extracellular neuraminidase [8]. These isolates, as 90% of the strains responsible for late onset disease [3], belonged to serotype III.

If the physiopathology of early onset GBS disease is well-documented, little is known about late or recurrent GBS infections. This case offers a novel hypothesis explaining how GBS can cause neonatal infection from 7 days to 3 months following delivery.

In most of the rare cases described [7, 9], there were no signs of maternal mastitis, indicating a silent maternal duct colonization. Moreover, National Committee of Hygiene guidelines do not systematically screen for GBS in the raw maternal milk supply [1]. These may be two reasons for the underestimation of maternal milk as a source of GBS infection. In the present case, genetic analysis affords evidence for maternal milk as the source of neonatal GBS infection. A circular process was hypothesized by Kotiw *et al* [7]. GBS initially colonizes the neonate's oropharynx mucosa from perinatal or other sources, infecting maternal ducts during breastfeeding. The organism multiplies in the milk ducts. As the microbial concentration increases in the milk, the infant is re-infected during breastfeeding. Mastitis may or may not be present [7]. However Olver *et al* described cases of GBS infection in preterm infants fed with maternal milk via nasogastric tube alone [9]. Prematurity is a recognized predisposing factor to GBS infection although breast milk transmission was also described in term infants [4].

In our unit, expressed mother’s milk is systematically pasteurized and frozen for conservation in our lactarium, and each specimen of milk is screened for

bacteria before administration to preterm infants. However, preterm infants might also receive raw, freshly-expressed breast milk from their mothers present in the unit. When a preterm infant falls clinically ill while the mother is breastfeeding, the mother's milk should be cultured to rule out or to document possible breast milk transmission. Mother's milk feedings should be suspended while providing banked milk pending culture result. If breast milk is positive for GBS, adequate antibiotherapy should also be prescribed for the mother. Byrne et al reported that it is possible to give the mother the opportunity to continue breastfeeding as desired ; she can be encouraged to maintain her milk supply by pumping and discarding milk until appropriate treatment is completed and negative breast milk cultures are obtained [4].

Asymptomatic excretion of GBS in breast milk may be an under-recognised cause of neonatal and recurrent infection. Recommendations should be established to prevent these infections, notably in the case of multiple births: 1/ treatment of both twins; 2 / recognition of the possibility of GBS breast milk infection in late onset or recurrent infection; 3/ suspension of breastfeeding upon suspicion of GBS breast milk infection in both of the children; 4/ search for GBS colonization in both mother and children.

However, it should also be noted that use of human milk in the intensive care nursery decreases the incidence of nosocomial sepsis [5] and breastfeeding should still be considered as the most appropriate nutrition for babies and preterm infants [1].

Conflict of interest statement

All authors, no conflict of interest.

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Figure 1

A. Pulsed-field gel electrophoresis (PFGE) of DNA restricted with *Sma*I of maternal and neonatal GBS isolates. All six isolates displayed identical PFGE patterns, showing their genetic relationship. Molecular weight (MW) ; maternal vaginal isolate (lane 1) ; Twin 1 CSF (lane 2) ; maternal raw breast milk isolate (lane 3) ; first (lane 4) and second (lane 5) Twin 1 blood culture isolates, and Twin 2 blood culture isolate (lane 6).

B. Ethidium bromide stain of 2% agarose gel showing multiplex PCR products for the GBS clinical isolates. Amplification of the tRNA gene clusters at the 3' end of rRNA operons produced a unique fragment of 1.2 Kb ; amplification *hyl*B gene produced a 0.3 Kb fragment, showing no IS*1548* insertion within the gene. This pattern was correlated to the invasive phylogenetic division I defined by Musser *et al*. in multilocus enzyme electrophoresis (MLEE) analysis [8, 12]. MW, molecular weight ; lane 1, maternal vaginal isolate ; lane 2, twin 1 CSF isolate ; lane 3, maternal raw breast milk isolate ; lanes 4 and 5, first and second twin 1 blood culture isolates.

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